REMARKS

The September 19, 2002 Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures states that the nucleotide and/or amino acid sequence disclosure contained in this application clearly fails to comply with the requirements of 37 C.F.R. §1.821 - §1.825 because sequences have been found in the subject specification. The Notice states that a substitute computer readable form must be submitted as required by 37 C.F.R. §1.825(e).

The Notice states that applicant must provide: 1) an initial or substitute computer readable form (CRF) copy of the "Sequence Listing"; and 2) a statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. §1.821(e) or §1.821(f) or §1.821(g) or §1.825(b) or §1.825(d).

In response, applicants, without conceding the correctness of the Examiner's position but to expedite prosecution of the subject application, enclose a computer diskette containing the sequence listing in computer readable form. Applicants attach hereto, as Exhibit B, a paper copy of the revised computer readable form of the sequence listing. Applicants attach hereto as Exhibit C a Statement in Compliance with 37 C.F.R. §1.821(f) certifying that the computer readable form contains the same information as the paper copy of the sequence listing attached as Exhibit B, and that the sequence listing does not contain any new matter.

In addition, applicants have hereinabove amended the specification to include references to the sequence identifier information (i.e., SEQ ID NO:) as required by 37 C.F.R. §1.821(d). Applicants attach hereto as Exhibit D a version of the amended paragraphs marked-up

to show the changes relative to the previous version thereof pursuant to 37 C.F.R. §1.121(b)(1)(iii). This amendment does not involve any issue of new matter. Therefore, entry of this amendment is respectfully requested.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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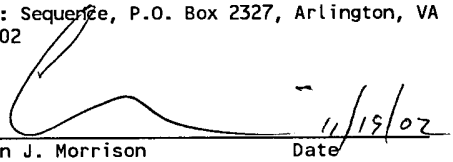
I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: U.S. Patent and Trademark Office BOX: Sequence, P.O. Box 2327, Arlington, VA 22202



11/19/02

Alan J. Morrison Date
Reg. No. 37,399

Marked-up Version of Amended Paragraphs

On page 12, lines 10-23:

Figure 14

Nucleotide sequences of Vβ8Dβ2.1Jβ2.6 junctions from the thymus of a 4 week old *Ku70-/-* mouse (SEQ ID NOS: 1-23). Products corresponding to Vβ8.1, Vβ8.2 or Vβ8.3 rearrangement with Jβ2.6 were cloned and sequenced. TCR Vβ8-Jβ2 joints were amplified by PCR (20, 27, 28) as described (see Fig. 3B). PCR cycling conditions were 94°C for 45", 68°C for 30", and 72°C for 30" (30 cycles). The band corresponding to Vβ8-Jβ2.6 was purified, reamplified for 20 cycles and then subcloned into the pCRII vector (Invitrogen). DNA was extracted from individual colonies and sequenced using the universal T7 and M13 reverse primers. Germline sequences are written in bold case, 'N' and 'P' denote nucleotides not present in the germline sequences.

On page 24, lines 25-32:

The genotype of the mice was first determined by tail PCR analysis which distinguishes endogenous from the targeted *Ku70* allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 μg genomic DNA; 0.6 μM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 24), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTG-GTGGTTGAGCC (SEQ ID NO: 26); 0.2 mM (each) dNTP; 1.5 mM $MgCl_2$ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min,

On page 25, lines 8-20:

To confirm that the disruption of *Ku70* produces a null mutation, Ku70 protein expression was measured by Western blotting using polyclonal antisera against intact mouse Ku70. The lack of Ku70 was also verified by a Ku-DNA-end binding assay (gel mobility shift analysis). Cell extracts were prepared and gel mobility shift assays were performed as described (22). Equal amounts of cellular protein (50 μg) from *Ku70+/+* (WT), *Ku70+/-*, and *Ku70-/-* mouse embryo fibroblasts were incubated with a ^{32}P-labeled double-stranded oligonucleotide, 5'-GGGCCAAGAATCTTCCAGCAGTTTCGGG-3' (SEQ ID NO: 27). The protein-bound and free oligonucleotides were electrophoretically separated on a 4.5% native polyacrylamide gel. Gel slabs are dried and autoradiographed with Kodak X-Omat film.

On page 27, lines 1-23:

To determine whether a null mutation in *Ku70* affects the recombination of antigen-receptor genes in T and B lymphocytes *in vivo*, we measured the immunoglobulin and T-cell antigen receptor (TCR) rearrangements by PCR. DNA from bone marrow was amplified with primers specific to immunoglobulin D-J_H and V-DJ_H rearrangements, and DNA from thymus was amplified with primers that detect V-DJ_β and D_δ-J_δ-rearrangement (20, 25-28). Oligonucleotides for probes and PCR primers specific to TCR Vβ-Jβ rearrangements and immunoglobulin D-J_H and V-DJ_H rearrangements are as follows. For TCRβ Vβ8-Jβ2 rearrangements (28): Vβ8.1: 5'-GAGGAAAGGT-GACATTGAGC-3' (SEQ ID NO: 28), Jβ2.6: 5'-GCCTGGTGCCGGGACCGAAGTA-3' (SEQ ID NO: 29), Vβ8 probe: 5'-GGGCTG AGGCTG ATCCATTA-3' (SEQ ID NO: 30). For $D_{\delta 2}$-

$J_{\delta 1}$ rearrangement (20, 27): DR6: 5'-TGGCTTGACATGCAGAAAACACCTG-3' (SEQ ID NO: 31), DR53: 5'-TGAATTCCACAG-TCACTTGGCTTC-3' (SEQ ID NO: 32), and DR2 probe: 5'-GACACGTGATACAAAGCCCAGGGAA-3' (SEQ ID NO: 33). For immunoglobulin D-J_H and V-DJ_H rearrangements (26): 5'D: 5'-GTCAAGGGATCTACTACTGTG-3' (SEQ ID NO: 34), V7183: 5'-GAGAGAATTCAGAGACAATC-CCAAGAACACCCTG-3' (SEQ ID NO: 35), VJ558L: 5'-GAGAGAATTCTCCTCCAGCACAG-CCTACATG-3' (SEQ ID NO: 36), J2: 5'-GAGAGAATTCGGCTCCCAATGACCCTTTCTG-3' (SEQ ID NO: 37), 5'IVS: 5'-GTAAGAATGGCCTCTCCAGGT-3' (SEQ ID NO: 38), 3'-IVS: 5'-GACTCAATCACTAAGACA-GCT-3' (SEQ ID NO: 39), and probe: a 6 kb EcoR I fragment covering the J region of mouse IgM.

On page 62, lines 23-34:

The genotypes of the mice were first determined by tail PCR analysis which distinguishes endogenous from the targeted Ku70 allele, and subsequently confirmed by Southern blot analysis. The PCR reaction contained 1 mg genomic DNA; 0.6 mM (each) of primers HO-2: GGGCCAGCTCATTCCTCCACTCATG (SEQ ID NO: 40), HO-3: CCTACAGTGTACCCGGACCTATGCC (SEQ ID NO: 25) and HO-4: CGGAACAGGACTGGTGGTTGAGCC (SEQ ID NO: 41); 0.2 mM (each) dNTP; 1.5 mM $MgCl_2$ and 2.5 U of Taq polymerase. Cycling conditions were 94°C for 1 min, 64°C for 1 min, 72°C for 1 min (30 cycles), followed by an extension at 72°C for 10 min. Primers HO-2 and HO-4 give a product of the targeted

On page 87, lines 17-25:

The genotype of the mice was determined by PCR which distinguishes endogenous from the targeted DNA-PKcs allele. PCR reaction contains 1 μg genomic DNA; 0.6 μM (each) of

primers MD-20: TATCCGGAAGTCGCTTAGCA-TTG (SEQ ID NO: 42); MD-21: AAGACGGTTGAAGTCAGAAGTCC (SEQ ID NO: 43); and POL-8: TTCACATACACC-TTGTCTCCGACG (SEQ ID NO: 44); 0.2 mM(each) dNTP; 1.5 mM $MgCl_2$ and 2.5U of Taq polymerase. Primers MD-20 and MD-21 give a product of wild type allele that is 264 bp; primers MD-20 and Pol-8 yield a product of the targeted allele that is 360 bp.

On page 88, lines 7-19:

For RT-PCR assay, total RNA was prepared from SV40 transformed lung fibroblast cells using Qiagen RNeasy kit (Qiagen Inc., Santa Clarita, CA). After digestion of contaminated genomic DNA by DNase I (Ambion, Austin TX), cDNA synthesis was carried out with the Superscript preamplification system (Gibco BRL, Gaithersburg, MD) according to the included protocol. PCR primers used for RT-PCR were MD-3: ATCAGAAGGTCTAAGGCTGGAAT (SEQ ID NO: 45), MD-5: CGTACGGTGTTGGCTACTGC (SEQ ID NO: 46) for amplification between exon 1 and 4 of DNA-PKcs , MD-28: CACTGAGGGCTT-TCCGCTCTTGT (SEQ ID NO: 47), MD-29: GCTCTTGTGCACGAATGTTGTAG (SEQ ID NO: 48) for PI-3 kinase domain, and GA-5: AGAAGACTGTGGATGGCCCC (SEQ ID NO: 49), GA-3: AGGTCCACCACCC-TGTTGC (SEQ ID NO: 50) for control GAPDH amplification.

On page 89, line 22- page 90, line 12:

T cell antigen receptor (TCR) and immunoglobulin recombination in T and B lymphocytes were measured by amplifying rearranged DNA fragments using PCR. Genomic DNAs were isolated from thymus, spleen and bone marrow (BM) from 4-to 9-week-old DNA-PKcs heterozygous (+/-), homozygous (-/-) mice and SCID

mice. Oligonucleotides for PCR primers and probes are as follow. For TCR_β $V_\beta 8$-$J_\beta 2$ rearrangement (16), $V_\beta 8.1$: GAGGAAAGGTGACATTGAGC (SEQ ID NO: 51), $J_\beta 2.6$: GCCTGGTGCCGGGACCGAAGTA (SEQ ID NO: 29), and $V_\beta 8$ probe: GGGCTGAGGCTGATCCATTA (SEQ ID NO: 52). For TCR_δ $D_\delta 2$-$J_\delta 1$ rearrangement, DR6: TGGCTTGACATGCAGAAAACACCTG (SEQ ID NO: 31), DR53: TGAATTCCACAGTCACTTGGGTTC (SEQ ID NO: 53) and DR2 probe: GACACGTGATACAAAGCCCAGGGAA (SEQ ID NO: 33). For TCR_δ $D_\delta 2$-$J_\delta 1$ signal joint (19), DR21: GTCATATCTTGTCCAGTCAACTTCC (SEQ ID NO: 54), DR162: GATGAGCCAGCTGGATGAGTAACAC (SEQ ID NO: 55), and DR161 probe: GCCCTCTAGCCATGACA TCAGAGC (SEQ ID NO: 56). For immunoglobulin $V_H 7183$-$J_H 4$ rearrangement(19), DR214: CGCGAAGCTTCGT GGAGTCTGGGGGA (SEQ ID NO: 57), DR217: GGGGAATTCCTGAGGAGACGGTGACT (SEQ ID NO: 58), and DR218 probe: ACCCCAGTAGTCCATAGCATAGTAAT (SEQ ID NO: 59). For control GAPDH amplification, same primers were used as RT-PCR experiment. Probe DNA for mouse GAPDH was purchased from Ambion Inc.(Cat.#7330, Austin TX). Amplified PCR products were resolved on 2% of agarose gel in 0.5x TBE, and transferred to Hybond N+ nylon membrane. Using radiolabeled oligonucleotide or DNA probes, PCR products were hybridized and visualized by autoradiography.